BEARING OF HETEROSIS UPON DOUBLE FERTILIZATION

DONALD F. JONES

(WITH THREE FIGURES)

The increase in development frequently observed in generations immediately following a cross in both plants and animals has been definitely correlated with heterogeneity of germinal constituents. The diverse effects resulting from this heterozygous condition have all been included in the one term heterosis (14). Various ways in which heterosis in plants may become visible have been described by different investigators. An increase in general vegetative luxuriance was first recorded by KÖLREUTER (11) as early as 1766. An increase in the facility of vegetative propagation has been shown for hybrids as well as an increased viability under adverse climatic conditions (GÄRTNER **9**, and references given there). DARWIN (7) gives numerous cases in which the rate of growth was increased by crossing. Both the time of flowering and maturing was hastened, as compared with the parents, in a large number of crosses, which also gave an increase in size.

To these many manifestations of the effects of heterozygosis COLLINS and KEMPTON (2) have added the fact that in maize the endosperm may also be increased in amount as an immediate result of crossing. By artificially pollinating maize with a mixture of two kinds of pollen, two visibly different kinds of seed were obtained upon the same ear (pistillate inflorescence) by taking advantage of xenia. The varieties of maize used in making these crosses differed among other characters in the color of the aleurone cells of the endosperm. A mixture of pollen of a variety with uncolored aleurone and of pollen of a variety with colored aleurone, when applied to the ear of a plant with uncolored aleurone, gave colored and uncolored seeds. In this way 11 ears were obtained, with the two kinds of seeds distributed at random. To produce the uncolored seeds, pollen from the same plant or another plant of the same variety was used. These seeds were then either selfed, or crossed with a closely related plant. The colored seeds, however, were the result of a cross with a different variety. The two kinds of seeds were separated and weighed. It was found that in all the 11 cases the out-crossed seeds exceeded the others in weight by percentages ranging from 3 to 21. Since the two genetically different kinds of seeds developed side by side in the same inflorescence, under as nearly the same conditions as it was possible to obtain, such increases in weight are surely significant.

That the increase in weight was a manifestation of heterosis and not merely the result of crossing a large seeded plant on a small seeded plant, was shown by the fact that where two varieties were used as pollen parents which differed in size of seed, one having seeds twice as large as the other, the crosses involving the large seeded plant showed no greater increases than the crosses in which the small seeded plant was used as pollen parent. In fact, the latter crosses gave rather greater increases. From this COLLINS and KEMPTON conclude that "the rate of increase bears no direct relation to the size of seed in the variety used as the source of pollen" (*loc. cit.* p. 11).

In the experiments of COLLINS and KEMPTON, reciprocal crosses were not made. Although the fact of increased endosperm development resulting from cross-fertilization is shown by the results reported, still more conclusive evidence has been obtained by the writer from reciprocal crosses in maize by the use of similar pollen mixtures. A number of crosses were made between types of maize previously selfed from 3 to 6 generations. These inbred strains were quite uniform and were derived originally from different cultivated varieties. Reciprocal crosses were made, not between individual plants, but between the different strains. All of the plants of each line, however, were descended from individual plants in the preceding generation and were genetically nearly identical.

Some of the strains had yellow, others white endosperm. Either way the cross was made the heterozygous seeds immediately resulting from pollination were light yellow, with a more or less distinct white or pale yellow cap. The pure yellow seeds in most cases could easily be distinguished from these heterozygous yellow seeds by their darker color and absence of the light colored cap. Mixtures of "white" and "yellow" pollen, therefore, applied either to a white or a yellow seeded plant, produced two distinct classes of seeds which could easily be separated. Some yellow strains were found which, when crossed by white, did not give heterozygous seeds clearly distinguishable from pure yellow. No such crosses were used in comparing the weights of selfed and crossed seeds. The reciprocal cross of yellow on white always gave yellow seeds clearly distinct from pure white, as would be expected.

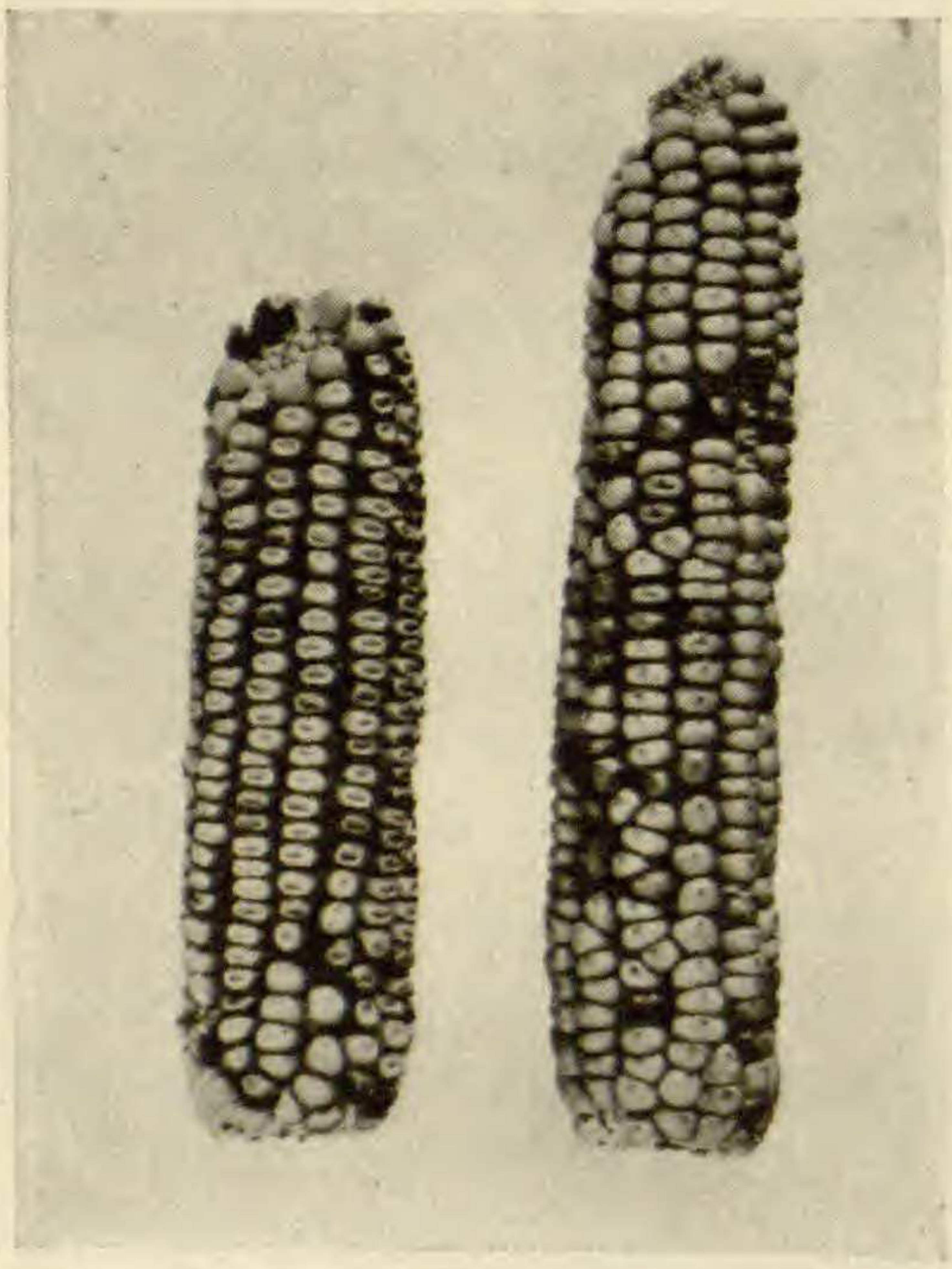

FIG. 1.—Two ears of maize with uncolored endosperm pollinated with mixture of "yellow" and "white" pollen, showing distribution of selfed and crossed seeds.

In all the ears resulting from the application of mixed pollen, the selfed and crossed seeds were distributed at random (fig. 1). Approximately equal quantities of pollen were used for each pollination, but, owing probably to the short viability of maize pollen (1), the two kinds were not always equal in their ability to fertilize. The proportion of selfed and crossed seeds, therefore, varied greatly. In some cases all the seeds were crossed, in others all selfed.

Twenty-four ears having both selfed and crossed seeds were obtained, and all gave an increase in average weight of the crossed seeds over the average weight of the selfed seeds, ranging from 5 to 35 per cent. The complete data will be published elsewhere, as these results were obtained in connection with a different investigation. A typical distribution of the weights of the selfed and crossed seeds in a reciprocal combination on two ears is shown in table I.[1]

TABLE I

DISTRIBUTION OF WEIGHTS OF SELFED AND CROSSED SEEDS OF MAIZE GROWN IN SAME INFLORESCENCES

Plant number; seeds grown in same inflorescence	Color of seeds	Condition of seeds	Weight of seeds in centigrams									Total number	Average weight	Increase	Percentage increase
			10	14	18	22	26	30	34	38	42				
14-10-4-6-4-7-26..	Yellow	Selfed				1	7	53	11	1		73	30.2±0.19		
14-10-4-6-4-7-26×20A-4-25-37.....	Light yellow	Crossed			1	0	3	12	72	90	10	188	35.9±0.16	5.7±0.25	18.9
20A-4-25-36........	White	Selfed	1	1	2	63	2					69	21.7±0.16		
20A-4-25-36×14-10-4-6-4-7-6....	Light yellow	Crossed				6	33	5				44	25.9±0.20	4.2±0.26	19.4

The crossed and selfed seeds on one of the ears shown in the table differ by 5.7 cgm. in average weight, a divergence which is 22 times the probable error. The reciprocally crossed ear produced seeds which differ by 4.2 cgm., or 16 times the probable error.

One ear with 5 crossed seeds and 328 selfed seeds gave the largest increase obtained in all the pollinations. The selfed seeds altogether averaged 37.3 cgm. in weight, while the 5 crossed seeds averaged 58.0 cgm. This is an increase of 55 per cent. Among the selfed seeds, however, were all the tip seeds, which were smaller in this ear (as is nearly always the case in maize) than the other seeds. The comparison is therefore unfair to the selfed seeds. Taking only the 10 seeds immediately adjacent to the 5 crossed seeds on the basal and apical sides the increase was still the largest obtained, 35 per cent. The crossed seeds were visibly larger, as shown in fig. 3.

The fact that the greatest increase was obtained where the proportion of crossed to selfed seeds was least, suggested that the heterotic seeds developed at the expense of the selfed seeds. An examination of all the data, however, showed that there was no significant correlation between the amount of increase and the

[1] A Jolly balance was fitted with scale and pointer so that the weights could be read off directly. A pan was constructed out of stiff paper in such a way that pressing the two ends together allowed the seeds to fall out through a slit in the bottom after weighing. This proved to be a great time saver. A magnifying glass helped in reading the scale (fig. 2).

proportion of the two kinds of seeds. Nearly as large increases were obtained where the number of crossed seeds greatly exceeded the selfed. These data obtained from reciprocal crosses fully substantiate the results reported by COLLINS and KEMPTON, and altogether show that CORRENS (5) was not wholly correct in stating that crossing does not immediately alter the size of seeds in maize.

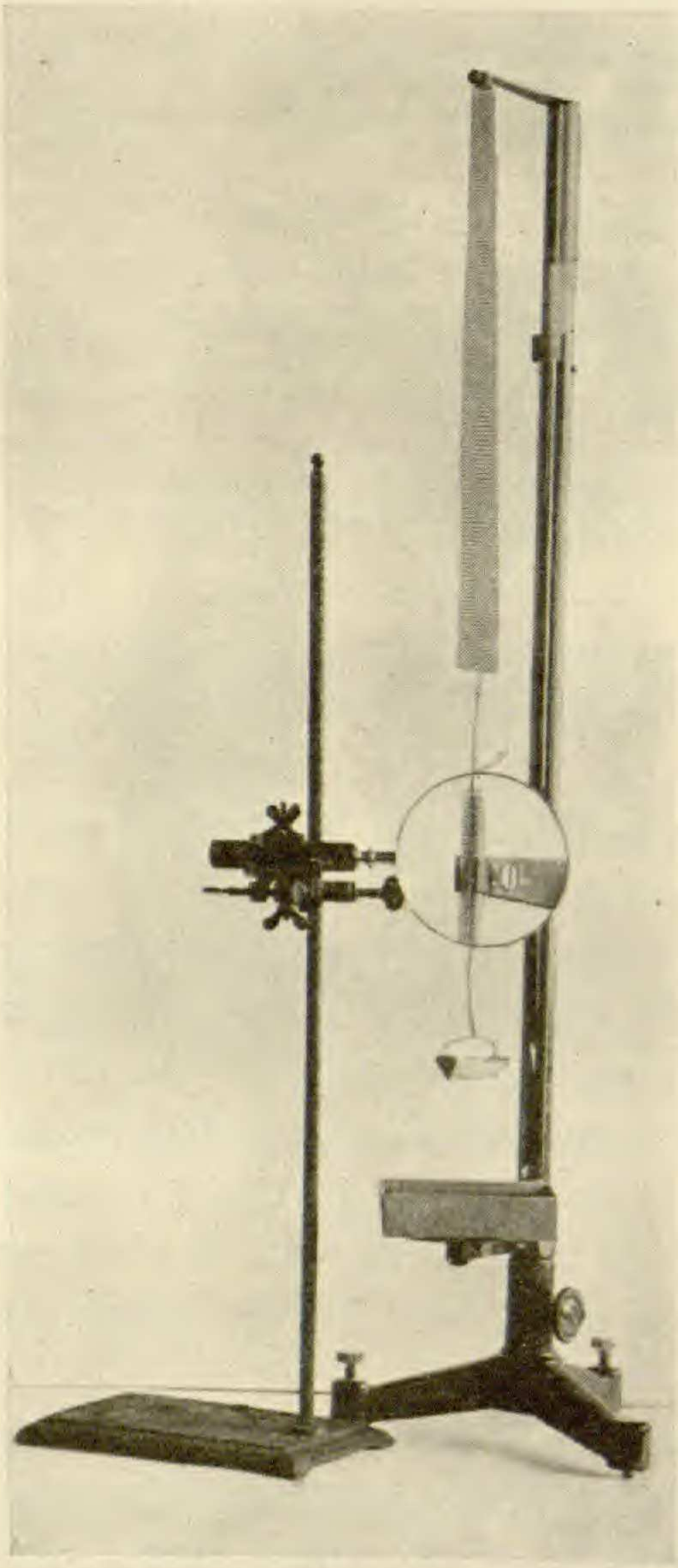

FIG. 2.—Machine used for weighing seeds.

So far as I know, maize is the only plant in which this manifestation of heterosis has been demonstrated. Since the main facts of xenia and heterosis as determined in maize do not differ essentially from the results obtained in other plants, there is every reason to suppose that increased endosperm development resulting from crossing is a phenomenon which may occur in many, if not all, other angiosperms where double fertilization takes place. Granted that this is so, what bearing do these facts have upon the puzzling problem of double fertilization in endosperm formation?

NĚMEC (13) has suggested, as a means of accounting for the origin of the process of endosperm hybridization, that it is an adaptation resulting in an alteration of the food supply to accord with the properties of a hybrid embryo. His own statements in regard to the matter are as follows:

In a case of hybridization, the embryo and the endosperm are assured the same physiological properties only when the endosperm fusion nucleus as well as the egg cell are fertilized by nuclei of the same properties, and this takes place in double fertilization. Double fertilization occurs even when the reserve substances entirely or to a great extent are put directly into the embryo, and we see that this is truly the case in many plants. In these plants nevertheless an endosperm at first develops and even results from double fertilization as

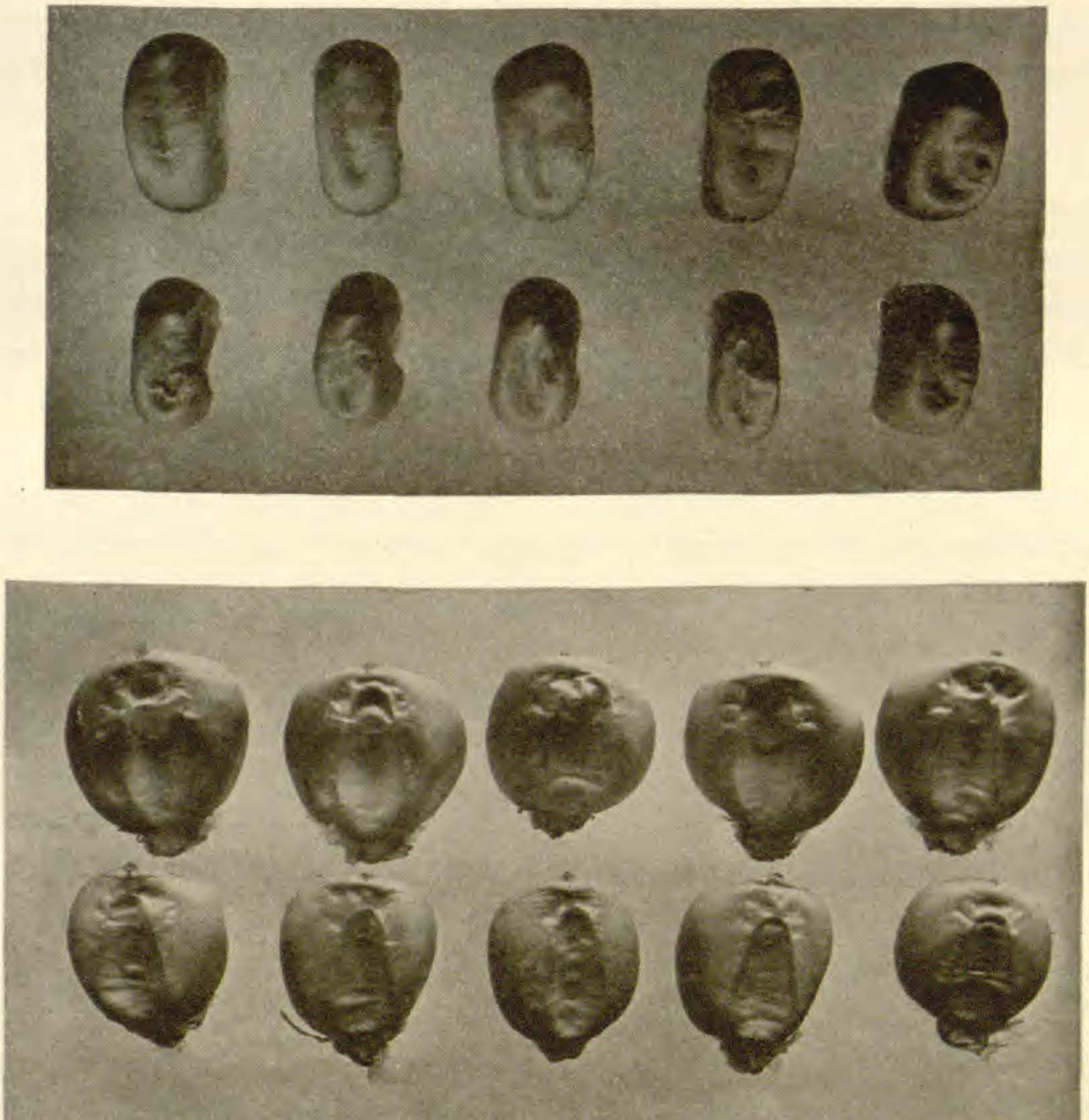

FIG. 3.—Crossed and selfed seeds from same inflorescence; top and side view of same seeds; crossed seeds above, selfed seeds below.

well, so it is possible that such plants exhibit a retention of a character and that in them the fertilization of the endosperms is only a useless relic. So from our standpoint double fertilization can be taken as an apparent adaptation in two ways: first, to stimulate endosperm development; second, to alter the endosperm physiologically to accord as far as is possible with the embryo. In this way a good nourishment of the seedlings by the endosperm material is assured (*loc. cit.* pp. 502, 503).

This is indeed an ingenious interpretation. Without endosperm hybridization an embryo resulting from a cross would be forced to depend entirely upon the kind of food supplied by one parent in its early stages of development, as is the case in all plants where double fertilization does not occur. It is conceivable that a wide cross might so alter the developing zygote that it would be less favorably nourished by food furnished by only one parent in the critical stages of its development. Hybridization of the endosperm, no doubt, may help to adapt the food to the requirements of the hybrid embryo more or less intermediate between the two parents. It would be still more serviceable in the rare cases of supposed merogony (3, 4, 8, 12). In these cases, however, nothing is known about the development of the endosperm, but what would be the nature of an embryo derived from such a wide cross that it would be retarded in its development because of an ill-adjusted food supply coming from one parent? Such an embryo would be so heterogeneous in its hereditary make-up that it would most likely not develop at all. In other words, the complexity permitted in the embryo would limit the diversity of hybridization before the dissimilarity in the composition of the food supplied by one parent could have any appreciable effect upon the development of the zygote.

To postulate the origin of endosperm hybridization as an adaptation having survival value, it is necessary to presuppose that it arose in plants which were naturally widely crossed. In such forms the effect of heterozygosis in increasing the amount of endosperm as shown in maize would, no doubt, have been operating. Hence, if it is feasible to account for the origin of double fertilization as an adaptation, it would seem more likely that such a process arose as a means of increasing the *amount* of food supplied to the embryo rather than as a method of adjusting its *composition* to the needs of the developing plant. In all probability both factors help in the early stages of a plant's development. Whether or not it is an adaptation, or whether either of these factors was concerned in the initiation of this puzzling process, I do not attempt to decide.

COULTER and CHAMBERLAIN (6) do not distinguish between the fusions of like nuclei and the fusion resulting in double fertilization. They say:

The development and structure of the endosperm of angiosperms is so much like that of gymnosperms that it seems easier to regard the various fusions as merely resulting in a stimulus to growth than to imagine a degenerate embryo assuming this particular development and structure (*loc. cit.* p. 183).

Considering double fertilization as an adaptation means that endosperm hybridization arose as a different process from that of nuclear fusion in which nuclei derived from one individual take part. Of course the union of like nuclei cannot be considered as a means of altering the food supply, so that NĚMEC's hypothesis has no bearing upon this phase of the problem. Neither can the union of like nuclei be a means of increasing the amount of food in the way that endosperm hybridization does, since heterosis, according to the hypothesis recently advanced by the writer (**10**), is not due to an indefinite physiological stimulus, but merely the result of bringing together the maximum number of growth factors showing partial dominance.[2]

If increased endosperm development is simply a manifestation of heterosis and as such can be put on a Mendelian basis, the process of endosperm hybridization, in so far as it arose as a means of either increasing the amount or altering the kind of food supply, is a phenomenon quite apart from the fusion of like nuclei. Moreover, if double fertilization came about as an adaptation, having occurred in cross-pollinated plants, it must have persisted as a process of no value, both in species which are now almost entirely self-pollinated, as well as in those which do not produce an appreciable amount of endosperm, as NĚMEC points out.

Whether or not heterosis can be removed entirely from the category of results due to indefinite "physiological stimulations," in which category the results of the fusions of like nuclei would still be, remains to be seen. Some interesting results obtained from wheat crosses have an important bearing on the question. Both G. F. FREEMAN[3] of the Arizona Experiment Station and K. SAX[3] of the Washington Experiment Station have obtained independently

[2] The two serious objections to the hypothesis of dominance as a means of accounting for heterosis previously advanced do not hold when the facts of linkage of hereditary factors are taken into consideration.

[3] Unpublished data.

crosses between two distinct types of wheat-macaroni (*Triticum sativum*, var. *durum*) and bread wheat (*Triticum sativum*, var. *vulgare*), which gave seeds much reduced in size and shrunken in appearance as the immediate result of cross-pollination. The smaller size and poor development of the seeds were due to the condition of the endosperm. The embryos were fully developed, however, and the first generation hybrid plants grown from these seeds were in some cases distinctly larger than either parent. This evidence of heterosis was shown in an increase in height of plant.[4] If this hybrid vigor were due merely to a physiological stimulation of cell division it would seem that the endosperm tissue would be stimulated in the same way and show an increased development. On the view that heterosis is due to a bringing together of the greatest number of different favorable growth factors, these results would be easier to understand if it be assumed that the aggregated factors were favorable to the growth of the first generation hybrid plant but not to the hybrid endosperm. Cases of this kind in wheat, which may be rare, however interpreted, would certainly argue against the origin of endosperm fertilization as an adaptive process.

CONNECTICUT AGRICULTURAL EXPERIMENTAL STATION
NEW HAVEN, CONN.

LITERATURE CITED

1. ANDRONESCU, D. I., The physiology of the pollen of *Zea Mays* with special regard to vitality. Publ. Depart. Agric. Kingdom of Roumania. 1915.
2. COLLINS, G. N., and KEMPTON, J. H., Effects of cross-pollination on the size of seed in maize. U.S. Dept. Agric., Circular 124. 1913.
3. ———, A hybrid between *Tripsacum* and *Euchlaena*. Jour. Wash. Acad. Sci. 4:114–117. 1914.
4. ———, Patrogenesis. Jour. Heredity 7:106–118. 1916.
5. CORRENS, C., Bastarde zwischen Maisrassen, mit besonderer Berücksichtigung der Xenien. Bibliotheca Bot. 53:1–161. 1901.
6. COULTER, J. M., and CHAMBERLAIN, C. J., Morphology of angiosperms. New York. 1912.
7. DARWIN, CHARLES, The effects of cross and self-fertilization in the vegetable kingdom. London. 1876.

[4] In some of the cases the increase in height was shown by actual measurements; in others, observation showed that the plants were at least as well developed as the parents.

8. DE VRIES, HUGO, Gruppenweise Artbildung. Berlin. 1913.
9. GÄRTNER, C. F., Versuche und Beobachtungen über die Bastarderzeugung im Pflanzenreich. Stuttgart. 1849.
10. JONES, D. F., Dominance of linked factors as a means of accounting for heterosis. Genetics **2**:466–479. 1917.
11. KÖLREUTER, J. G., Dritte Fortsetzung der Vorläufigen Nachricht von einigen das Geschlecht der Pflanzen betreffenden Versuchen und Beobachtungen. 1766. Reprinted in OSTWALD'S Klassiker der exakten Wissenschaften. No. 41. Leipzig. 1893.
12. MILLARDET, A., Note sur l'hybridation sans croisement ou fausse hybridation. Mém. Soc. Sci. Phys. Nat. Bordeaux **4**:347–372. 1894.
13. NĚMEC, B., Das Problem der Befruchtungsvorgänge. Berlin. 1910.
14. SHULL, G. H., Duplicate genes for capsule-form in *Bursa bursa-pastoris*. Zeitsch. Ind. Abst. u. Vererb. **12**:97–149. 1914.